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Variation of barrier permeability for albumin and immunoglobulin G influx into cerebrospinal fluid

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Abstract

Background: The aim of the present study was to analyze variations in permeability of albumin and immunoglobulin G (IgG) influx into cerebrospinal fluid (CSF) in a clinical setting.
Methods: In a retrospective intra-individual comparison of CSF samples, we used the IgGIndex and its constituents to indicate alterations in IgG/albumin permeability.
Results: We found altered IgGIndices in 25/64 patients (range –25% to +44%), with differently altered QAlb and QIgG values (–69% to +549%), unaltered IgGIndices in a further 25/64 patients with equally altered QAlb and QIgG values (–46% to +107%), and no parameter alteration in 14/64 patients. Parameter alterations in 25/64 patients indicated that permeability of albumin was changed to different extents than for IgG. It changed in the same direction in 20/25 patients, and the opposite in five patients. In further 25/64 patients, equal QAlb and QIgG alterations indicated equally altered permeabilities and/or altered efflux of the proteins. In 14/64 patients, no alteration in permeability or efflux was seen.
Conclusions: Results revealed surprisingly variable intra-individual changes in permeabilities for albumin and IgG in pathologic as well as normal CSF. Differing changes in permeability indicate that the diffusion paths of the two proteins may react to disturbances independently of each other. The details of the influx permeability for albumin and IgG into CSF illustrate the prospect of a more comprehensive insight into the protein exchange between blood and CSF.
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Keywords: albumin; barrier permeability; cerebrospinal fluid (CSF); immunoglobulin G (IgG); intra-individual variation.

Introduction

Two main functions seem to characterize albumin and immunoglobulin G (IgG) exchange between blood and cerebrospinal fluid (CSF): Influx by diffusion depending on permeability and concentration gradients, and efflux together with CSF depending on pressure gradients (1–4). The traditional barrier parameter ''albumin quotient'' (QAlb = [CSF albumin]/[serum albumin]) expresses the combined effects of influx and efflux on the CSF concentration of albumin. However, influx and efflux of plasma proteins differ and vary on an individual basis (1–7) and cannot be differentiated with the albumin quotient. To understand details of exchange, it is necessary to measure the separate functions. At present, they have found little attention and appropriate absolute measures have not been established. We used the IgGIndex (IgGIndex = QIgG/QAlb) and its constituents as relative measures for a pilot study of intra-individual variation of influx permeability for the two proteins into CSF.

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Materials and methods

The study sample was extracted from our CSF data bank for the past 12 years. After exclusion of CSFs with oligoclonal bands, >1000 erythrocytes/μL, or collected after albumin or IgG infusion, we identified 126 patients who had two lumbar punctures (LP). Samples were usually evaluated within 30 min of collection with cell counting in a counting chamber and cell preparation with a cytocentrifuge and appropriate staining. Albumin and IgG in CSF and a simultaneous serum sample were quantified in the same run using a Behring nephelometer (Dade Behring, Marburg, Germany). Isoelectric focusing was performed using macro polyacrylamide gel (MacroPAG) with silver stain to identify relevant intrathecal IgG synthesis. External inter-laboratory assessments showed mean absolute measurement imprecision for albumin and IgG concentrations below 5%, and good results for isoelectric focusing. Collection and analytic methods remained unchanged throughout the sampling period. To reduce the risk of a disturbed blood-CSF protein steady state being caused by different blood-CSF equilibration times of both proteins and plasma protein interaction (1, 2, 8), we excluded large changes of plasma protein levels with a presumably higher risk of disturbance: in 126 patients with intra-individual variations in serum albumin concentrations by –32% to +78%, and in IgG concentrations by –43% to +48%, we chose ±10% as the cut-off value. We selected 64 patients with lower variation and presumably a lower risk of steady state disturbance for the study sample (Table 1). To reduce false ratings we sorted parameter changes. Changes in the IgGIndex or QAlb or QIgG values by >+10% or <–10% were chosen to indicate a probable parameter alteration, changes by ≤+10% and ≥–10% were used to indicate no alteration.

The IgGIndex was used as a parameter of permeability. Provided that intrathecal IgG synthesis is excluded, it approximates the permeability for IgG/albumin on all influx paths between blood and CSF, as expressed by the following calculation: inserting the equations of [CSF albumin] = [serum albumin] × albumin permeability/CSF turnover and of [CSF IgG] = [serum IgG] × IgG permeability/CSF turnover

Table 1 Demographics of study patients.

	Initial LP	Follow-up LP
QAlb$\times 10^{-3}$	11.6 (1.9–160)	12.2 (1.3–194)
QIgG$\times 10^{-3}$	6.6 (1–123)	6.5 (0.6–131)
IgGIndex	0.48 (0.33–0.86)	0.48 (0.38–0.67)
Number of male/female patients	31/33	
Age, years	51 (15–85)	
Time span between LPs, days	460 (1–3425)	
Number of patients with respective diagnoses	14 neuropathy, 12 encephalitis, 7 meningitis, 6 hydrocephalus, 6 headache, 4 polyradiculitis or variant, 3 myelitis, 3 radiculopathy, 2 dementia, 2 seizure, 1 brain infarction, 1 brain edema, 1 brain lymphoma, 1 hypoxia, 1 psychosomatic disease	

Values are expressed as mean and (range). LP, lumbar punctures.

(9, 10) into the definition of the IgGIndex=[CSF IgG]/[serum IgG]$\times$[serum albumin]/[CSF albumin] leads to IgGIndex=IgG permeability/albumin permeability. Intra-individual comparison of IgGIndices and its constituents may then be used to describe changes in IgG/albumin permeability: altered IgGIndices would indicate different permeability changes for IgG and albumin, whereas unaltered IgGIndices with equally altered QAlb and QIgG values would indicate equal changes in albumin and IgG permeability and/or changes in protein efflux. The latter is based on the concept that both albumin and IgG flow as solutes in the CSF and out of the thecal space. No change in the IgGIndex and the QAlb and QIgG values would indicate no change in permeability and efflux of the proteins. Further deductions and limitations will be discussed below. Statistical tests were not applied because of selection bias and restricted sample size. All sampling of CSF and serum was indicated clinically. Patients consented in written form to further evaluation of their data. The Local Ethics Committee approved the study.

Results

The IgGIndex was altered in 25/64 patients by $<-10\%$ or $>+10\%$, indicating likely changes in IgG/albumin permeability (Table 2, Figure 1). Respective QIgG and QAlb values changed differently, indicating differing patterns of change in albumin and IgG permeabilities. The IgGIndex decreased in 11/25 patients, with QIgG decreasing more than QAlb in six patients, QIgG increasing less than QAlb in four patients, or QIgG decreasing and QAlb increasing in one patient. The IgGIndex increased in 14/25 patients, with QIgG increasing more than QAlb in six patients, or QIgG decreasing less than QAlb in four patients, or QIgG increasing while QAlb decreased in four patients. The remaining 39/64 patients showed IgGIndex changes that were $\geq -10\%$ and $\leq +10\%$. QIgG and QAlb values changed in 25/39 patients by $<-10\%$ or $>+10\%$, respectively, indicating equal changes in permeability and/or changes in efflux of IgG and albumin. In 14/39 patients, QIgG and QAlb values changed by $\geq -10\%$ and $\leq +10\%$, suggesting unaltered permeability and efflux. The changes in these parameters occurred in pathologic and normal CSF, and were not consistent with respect to clinical diagnosis, clinical course, and CSF cell counts. A few peculiar constellations may be mentioned. All seven meningitis patients had IgGIndex changes of $<-10\%$ or $>+10\%$, indicating changes in permeability (Figure 1). Of 22/64 patients who had worsened clinically, three had decreased QIgG and QAlb values (one patient each with either

Table 2 Intra-individual IgGIndex, QIgG, and QAlb changes in 64 patients between initial LP1 and follow-up LP2.

Sorting of parameters by changes in	n	Changes between LP1 and LP2 (%) of		
		IgGIndex	QIgG	QAlb
1. IgGIndex by $<-10\%$	2	−22 to −25	−44 to −69	−27 to −59
	4	−13 to −22	−13 to −26	−2 to −5
	2	−15 to −23	+24 to +52	+46 to +96
	2	−13 to −21	+1 to +6	+21 to +28
	1	−18	−11	+12
IgGIndex by $>+10\%$	3	+12 to +32	+32 to +549	+18 to +401
	3	+11 to +24	+19 to +25	+1 to +8
	4	+12 to +33	−15 to −53	− 34 to −64
	2	+36 to +44	+17 to +20	−12 to −19
	2	+12 to +20	+1 to +11	−8 to −11
2. IgGIndex by $\geq -10\%$ $\leq +10\%$ and QAlb and/or QIgG by $<-10\%$	13	−8 to +10	−11 to −44	−11 to −46
or QAlb and/or QIgG by $>+10\%$	12	−8 to +9	+19 to +107	+11 to +103
3. IgGIndex by $\geq -10\%$ $\leq +10\%$ and QAlb and QIgG by $\geq -10\%$ $\leq +10\%$	14	−10 to +9	−10 to +10	−10 to +10

1, probable and differing changes in IgG and/or albumin permeability; 2, probable and equal changes in IgG and albumin permeability and/or changes in efflux; 3, no probable change in permeability or efflux; n, number of patients. Changes are expressed by quotients of IgGIndex, QIgG, and QAlb values of LP2/LP1 in %, e.g., −13, decrease by 13%. LP, lumbar punctures.

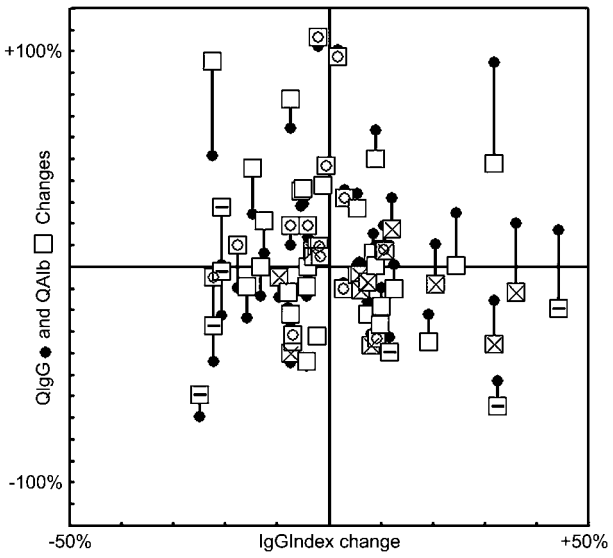

Figure 1 Range of intra-individual IgGIndex, QIgG, and QAlb changes in 63 patients between initial LP1 and follow-up LP2.
Outlier values for one patient are not shown (changes of QAlb by +401%, QIgG by +549%, IgGIndex by +29%). Parameter changes indicate changes in permeability and/or efflux of albumin and IgG. The specific diagnoses are varied among parameter changes, possibly due to selection bias. (Open square = % change in QAlb; solid circle = % change in QIgG; intra-individual pairs of QAlb and QIgG symbols are connected by a vertical line; corresponding X-value = % change in IgGIndex. Signs inside QAlb squares specify diagnoses: open circle, neuropathy; cross, encephalitis; horizontal bar, meningitis. Definition of changes in methods and Table 2).

brain lymphoma, Miller-Fisher syndrome, or hydrocephalus) suggesting decreased permeability and/or increased efflux; of 15/64 patients with clinical improvement, two patients, one with basilar migraine and one with brain hypoxia had increased and pathologic QIgG and QAlb values suggesting increased permeability and/or decreased efflux. CSF cell counts changed in a similar manner to clinical course or remained normal.

Discussion

The finding that 50/64 patients had probable alterations in parameters, vs. 14 patients with no alteration, suggests frequent changes in influx/efflux functions for albumin and IgG between blood and CSF. One should remember that the frequencies observed probably reflect patient selection, differing LP indications and time spans between LPs, as well as limitations of our parameters.

Our parameters assess compound effects of changes in influx permeability, and in efflux of albumin and IgG in the lumbar CSF. Influx permeability includes permeability for diffusion across choroidal microvessel endothelium and epithelium into the CSF, for diffusion across further central nervous system (CNS) endothelia directly or via the brain into the CSF, and for barrier-free diffusion across circumventricular organs. Efflux includes the flow of albumin and IgG within the CSF and out of the thecal space, and Fc receptor mediated IgG transport from the CNS into the blood (11–14). Flux quantities are not known in detail. The large surface area of the microvessel endothelia suggests that albumin and IgG diffusion should constitute the major influx. The large serum-CSF concentration gradients of albumin and IgG imply a much stronger efflux of both proteins compared with influx. This suggests that the major efflux occurs with bulk flow of CSF. Outflow routes via arachnoid granulations, lymph, or other paths are being debated (13). We assume that this concept of albumin and IgG influx through microvessel endothelia and efflux by bulk flow conforms with conditions of the parameter calculations we used (9, 10). There are further aspects to consider: 1) our parameters do not detect changes in efflux occurring simultaneously with differing changes in permeability for albumin and IgG. Efflux changes cause equal shifts in QAlb and QIgG values which preserve the differences in the permeability-related changes in QAlb and QIgG values. If large, the shifts may change the IgGIndex. Some permeability changes may thus be under- or over-estimated in our analysis. Efflux changes will be underrated. 2) Changes in permeability may be restricted locally (6). CSF mixing may dilute effects of such changes and lead to under-estimation of changes in permeability. 3) Our sample showed frequent differences in changes in QIgG and QAlb values indicating that changes in permeability of IgG and albumin often differ from each other. Equal changes in permeability cannot be differentiated from efflux changes by our parameters. To our knowledge, they have not been documented. To what extent they occur remains to be seen. 4) The effects of flow of IgG and albumin from brain tissue into the CSF are included in our permeability parameter; if the flow is large it may partly alter interpretations. 5) Fc receptor mediated IgG transport out of the CNS, if found to be a major IgG efflux, would also partly alter the interpretation. 6) Our parameters compare influx/efflux states of two CSF samples collected at different times. In the interim, multiple physiologic or pathologic factors may have caused changes in permeability and/or efflux. Our data, therefore, show the sum effect of changes, if they persisted up to the second sample. This may be more relevant for long-time intervals between LPs, and in chronic disease.

We consider the primary finding of this study to be the demonstration that intra-individual changes in IgG and albumin permeability were variable and different. This suggests that influx paths for the two proteins may react independently to disturbances. It also shows that an increased IgGIndex may reflect changes in IgG and/or albumin influx permeability and not necessarily intrathecal IgG synthesis. The parameters indicated that changes in permeability, and probably efflux, occurred in pathologic and normal CSF. This suggests that they may be pathologic as well as normal phenomena. Changes in influx, as well as possibly in efflux, were distributed among diagnoses which may express selection bias. Changes in perme-

ability in all meningitis patients may reflect widespread affection of barrier tissue. Some unusual combinations of parameter changes and course of disease point to an unusual possibility of decreasing permeability and/or increasing efflux with worsening of disease, and reverse changes occurring during improvement.

Our results showing differing and rather variable changes in albumin and IgG permeability, changes in permeability and probably efflux in normal CSF, and unusual influx and/or efflux changes were unexpected. They provide a more detailed insight into the exchange in albumin and IgG between blood and CSF. For a more precise analysis, absolute and practical measures of separate influx and efflux functions are needed.

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